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Diversity of Arbuscular Mycorrhizal Association with Some Plants of Abandoned Cropland in J. P. University Campus, Chapra Bihar.

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***Abstract** : The present study was to analyse the mycorrhizal diversitry in 12 plants of J. P. University Campus, Chapra. Arbuscular mycorrhizal fungal colonization ranged from 44.4 – 100 % . The highest infection was found in Parthenium hysterophorus L. and lowest in Ricinus communis L . AM fungal spore population with a range of 10 – 155 in 100 g of rhizosphere soils was detected. The maximum spore population was observed in the species , Clerodendrum infortunatum Linn. (155/ 100 gm of soil) and minimum in Citrus lemon (L.) (10/ 100 gm of soil). Totally 25 AM fungal species were isolated which belongs to five genera (Acaulospora, Entrophospora, Glomus, Scutellospora and Sclerocystis) and among them Glomus was dominant genera. The maximum relative abundance (RA) was recorded in Glomus intraradices (33.3 %) and minimum RA was recorded in Acaulospora delicata, Acaulospora morrowiae etc (0.16%). Highest isolation frequency (IF) was recorded for Glomus fasiculatum (100%) and lowest IF was recorded for A. delicata, A. denticulata etc (8.33%).*

***Keywords** : Arbuscular mycorrhiza, Root colonization, Relative abundance, Isolation frequency, Spore density.*

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I. Introduction

A symbiotic association of a fungus and roots of higher plants was discovered by Franciszek Kamienski, a polish mycologist in later Frank (1985) coined the term " Mycorrhiza" to this association . Mycorrhiza belonging to most commonly occurring soil microorganisms of the world is considered as a fundamental part of the plant, as 95 % of all plant species could not servive in nature without it. Arbuscular mycorrhizal (AM) fungi are obligate biotrophs feeding only on the products of photosynthesis of their alive plant hosts. AM fungi consist of intra and extraradical structures. The intraradical structures are arbuscules, vesicles and intraradical hyphae. The extraradical structures are extraradical hyphae, spores and auxillary cells. Haustorium like arbuscules are the main sites of nutrient exchange between a plant host and a fungus.They are formed within the cells of the inner root cortex(Mosse and Hepper 1975) and are indicators of active mycorrhizae. Association of AM fungi with plants help in increased plant growth (Aneesa et al.2010). Arbuscular mycorrhizae (AM) are regarded as a mutualistic association in which plant provides the fungus with assimilates in exchange for mineral nutrients and water. (Smith and Read 1997). Mycorrhizal diversity is high in forest when compared to other areas (Shruti et al. 2009). In natural communities, approximately 80% of higher plants are obligatorily dependent on fungal associates and 18% typically non mycorrhizal (Trappe, 1987). This is in contrast to the antagonistic interactions of plants and pathogenic fungi, with defense mechanism of arbuscular mycorrhizal fungal relationship with plants which can increase the growth of plants by enhancing phosphate uptake mainly and perhaps the other minerals such as K, Fe, Cu, Ca and Zn (Lakshman, 2009). VAM fungi takes roles in maintaing ecosystem processes by promoting plant fitness through a range of mechanism (Brundrett et al. 2008) ; protecting plant host from soil pathogens (Smith 2008) and improving soil structures enhancing water and nutrient uptake (Smith et al. 2010) ; increase the efficiency of fertilizers use and plant growth (Douds et al. 2010). Seasonal variation in spore density is frequently observed (Singh and Varma 1985 ;Sylvia 1986). Mycorrhizal fungi can potentially influence soil aggregation at different levels, namely plant communities, plant roots (individual host) and effects mediated by the fungal mycelium itself (Rillig et al. 2006). Mycorrhizal fungi also produce enzymes, auxins, cytokinins, vitamins and other substances that increase rootlet size and longivity (Dixon 1990).

Therefore , the aim of the present study was to examine the abundance of arbuscular mycorrhizal fungi extent of root colonization, isolation frequency and species diversity of AMF and to identify the dominant AM fungal species found therein.

II. Study Area

The present study was conducted during 2015 – 2017 in rainy season in the nine year old abandoned cropland of J. P. University , Chapra Campus which is spread in about 240 ha land . The study area is situated between $25^0 36^{'}$ - $26^0 15^{'}$ N lat. and $84^0 24^{'}$ - $85^0 15^{'}$ E long. The maximum temperature values ranged from 15.4^0 to 44.5^{0C}.

III. Materials And Methods

3.1 Root sampling - In the months of rainy season of 2015 – 2017 fine roots of plants growing in the campus of J. P. University were collected from different plants such as Calotropis gigantea (L.)Ait. , Ziziphus mauritiana Lamm., Croton sparciflorus(L.), Parthenium hysterophorus L. , Dalbergia sissoo Rox. Ex Dc. etc . Roots were collected randomly from a depth of 0- 30 cm . After bringing these plant samples to laboratory the roots were separated and further processing was done.

3.2 Estimation of root colonization - Roots were washed throughly to remove attached soil particles. The cleaned roots were cut into 1 cm long piece and were fixed in formalin acetic acid (FAA) according to the procedure described by Phillips and Hayman (1970) . The roots were boiled in 10% KOH for 1 hr, acidified with 5N HCI and stained for 24 hr with 0.5 % tryphan blue. Each root was divided into 12 1cm long segments, which were then cleaned, stained and were arranged on slides. The slides were observed under compound microscope to score for any structures associated with mycorrhizal fungi like hyphae, vesicles, arbuscules, or hyphal coil in each segment. The percentage of AM fungal colonization was assessed by using the formula :

*** Percentage of colonization** = Number of root segments infected x 100
Total number of root segments observed

3.3 Spore extraction -Separation of AM fungal spores from rhizospheric soil of each plant was done by using wet sieving and decanting method proposed by Gerdmann and Nicolson (1963) from the 100 gm of soil sample. Soil samples were collected randomly using three replicates . All the samples were sieved (< 2mm mesh size) to remove stones, coarse roots and other litter, and fine roots were collected from each sample. The root soil mixture was vigorously mixed with a glass rod for 30 seconds. The suspension was passed through 250µm, 150µm, 98µm and 75µm seives. The material remaining on the sieve was washed into beakers. After settlement of the heavier particles , the supernants was filtered through gridded filter papers. Each filter paper was spread on to a glass plate and scanned under stereo microscope (Olympus SZ2-ILST). Intact and crushed spores were counted. AM fungal spores from the filter paper were picked up using a wet needle and mounted in Polyvinyl alcohal lactophenol (PVLG) on a glass slide and identified under a compound microscope (Olympus BX41) and photographed (Nikon eclipse 200). ldentification was based on spore morphology and sub cellular characters (Schenck and Perez 1990).

3.4 Relative abundance and Isolation frequency of occurrence of AM fungi – Relative abundance of occurrence of AM fungi was calculated dividing the number of soil samples that possess spores of particular species with the total number of soil samples screened and multiplied by 100. Isolation frequency was calculated dividing the number of soil samples possessing spores of a particular species with the total number of soil samples analyzed and multiplied by 100.

$$RA = \frac{\text{Number of spores of a species / genus}}{\text{Total number of spores in all soil samples}} \times 100$$

$$IF = \frac{\text{Number of soil samples possessing spores of a particular species}}{\text{Total number of soil samples analyzed}} \times 100$$

IV. Results

The rate of AM fungal colonization in roots and endo- mycorrhizal spore diversity in rhizosphere of herbaceous vegetation showed wide range of variation in J. P. University Campus , Chapra Bihar. Colonization was characterized by the presence of hyphae, arbuscules, vesicles and hyphal coil. AM fungal colonization varied with species and the situation of the occurrence. The vegetative plant species along with their AMF characterizations are presented in Table 1. Percentage colonization was maximal for Parthenium hysterophorus L. and Lantana camara Var. (100%) and minimal for Ricinus communis L. (44.4 %) . Both Arum and Paris type morphologies were observed. Twenty five AM fungal species of five genera viz, Glomus , Acaulospora, Scutellospora, Sclerocystis and Entrophospora were recovered from rhizosphere soils of study sites. Glomus (14 species) was the dominated genus followed by Acaulospora (8 species), Scutellospora (1 species), Sclerocystis (1 species) and Entrophospora (1 species). Glomus species has been recorded as dominant mycorrhizal genus (Nisha et al. 2010). The highest spore density was recorded in the plant species of Clerodendrum infortunatum Linn. (155/ 100 gm of soil) belongs to the family Lameaceae. The lowest spore density was recorded in Citrus

lemon (L.) (10/ 100 gm of soil) belongs to the family Rutaceae. Twelve plant species of J. P. University of Chapra, Campus were analyzed for mycorrhizal density, diversity , Relative frequency (RA %) , Isolation frequency (IF %) etc in the present study. The major population of Glomus species has also been recorded followed by Acaulospora, Entrophospora, Sclerocystis and Scutellospora (Koul et al.2012 ; Nisha et al. 2010 ; Parwaara et al. 2012; Peterson et al. 1985; Phillips et al. 1970; Porter et al. 1987; Rajkumar et al. 2012).The maximum AM diversity recorded in Ricinus communis L. (13) and minimum AM diversity was recorded in the plant species of Citrus lemon (L.) (2) (Table1). The maximum relative abundance (RA) was recorded in Glomus intraradices (33.38 %) and minimum RA was recorded in Acaulospora delicata, Acaulospora morrowiae, Acaulospora undulata , Glomus aureum, Glomus multicaule and Scutellospora reticulata (0.16%) . Highest isolation frequency (IF) was recorded for Glomus fasiculatum (100%) and lowest IF was recorded for A. delicata, A. denticulata, A. morrowiae, A. scrobiculata, A. undulata, G. aureum, G. multicaule. G. pustulatum, Scutellospora reticulata and Sclerocystis rubiformis (8.33%) (Table 2). Higher numbers of Glomus species were recorded in the present study . These observations are in consistence with early reports (Mosses,1990).

Table: l AM association with roots and AM Spores present in rhizospheric soil

Sr no	Plant name	Colonization (%)	Spore density per 100 gm soil	AM diversity
1	Calotropis gigantea (L.) Ait.	97.7 ± 4.8 ±2.8	78.33	A. lae, A. spinosa, G. clar, G. fasi, G. intr, G. macro
2	Citrus lemon (L.)	72.2 ± 4.8 ± 2.8	10	G. fasi, S. reti
3	Croton sparciflorus (L.)	94.4 ± 9.64 ± 5.5	113.33	G. clar, G. etuni, G. fasi, G.geo, G. intr, G. macro, G. moss, G. multi, S. rubi
4	Azadirachta indica A. Juss.	63.8 ± 4.7 ± 2.7	88.33	G. clar, G. fasi, G. intra
5	Clerodendrum infortunatum Linn.	77.7 ± 20.9 ± 12.10	155	G. clar, G. fasi, G. intra
6	Ziziphus mauritiana Lamm.	72.2 ± 4.8 ±2.8	93.33	Entro, G. agg, G. clar, G. clarum, G. fasi, G. intra, G. moss
7	Parthenium hysterophorus L.	100 ± 0 ± 0	125	Entrpo, G. agg, G. clar,G. fasi, G. intra, G. lae
8	Dalbergia sissoo Rox. Ex Dc.	63.8 ± 4.7 ± 2.7	73.33	G. agg, G. clar, G. fasi, G. intr, G. macro, G. moss G. hoi
9	Phoenix dactylifera L.	47.16 ± 17.3 ± 10	38.33	A. lae, G. clar,G. dimor, G. fasi, G. intra, A. (unidentified)
10	Lantana camara Var.	100 ± 0 ± 0	48.33	A. deli , A. undu, A. morr, A. (unidentified) , G. aur, G. clar, G. etun, G. fasi, G. intra, G. macr, G. hoi
11	Ricinus communis L.	44.4 ±4.84 ±2.8	86.66	A. dent, A. scro, A. spi, G. agg, G. clar, G. dimor, G. etun, G. fasi, G. geo, G. hoi, G. intr, G. moss, G. pust
12	Psidium guajava L.	52.7 ±4.7 ± 2.7	128.33	G. cla, G. claru, G. dimor, G. etu, G. fasi, G. geo, G. intra, G. moss

A.spi - Aculospora spinosa, A. dent- Aculospora denticulata, A. lae- Aculospora laevis, A. morr – Aculospora morrowiae, A. scro- Aculospora scrobiculata, A. Spi – Aculospora spinosa, A. undu- Aculospora undulata, A. (uni)- Aculospora (unidentified), Entrophospora, G. agg – Gloumus aggegatum, G. aur- Glomus aureum, G. clar- Glomus claroideum, G. claru – Glomus clarum, G. dimor- Glomus dimorphicum, G. etu –

Glomus etunicatum, G. fasi- Glomus fasiculatum, G. geo- Glomus geosporum, G. hoi- Glomus hoi, G. intra- Glomus intraradices, G. moss- Glomus mosseae, G. macr- Glomus macrocarpum, G. multi- Glomus multicaule, G. pust- Glomus pustulatum, S. reti- scutellospora reticulata, S. rubi- Sclerocystis rubiformis.

Table : ll Relative abundance (RA%) and Isolation frequency (IF %) of AM fungi.

Sr no	AM Fungal Species	RA (%)	IF(%)
1	Acaulospora delicata	0.16	8.33
2	Acaulospora denticulata	0.16	8.33
3	Acaulospora laevis	1.30	25
4	Acaulospora morrowiae	0.16	8.33
5	Acaulospora scrobiculata	0.32	8.33
6	Acaulospora spinosa	0.81	16.66
7	Acaulospora undulata	0.16	8.33
8	Acaulospora (unidentified)	0.49	16.66
9	Entrophospora	1.14	16.66
10	Glomus aggrgatum	2.94	33.33
11	Glomus aureum	0.16	8.33
12	Glomus claroideum	13.74	91.66
13	Glomus clarum	0.32	16.66
14	Glomus dimorphicum	0.98	25
15	Glomus etunicatum	6.21	33.33
16	Glomus fasiculatum	26.84	100
17	Glomus geosporum	1.14	25
18	Glomus hoi	0.65	25
19	Glomus intraradices	33.38	91.66
20	Glomus macrocarpum	1.80	33.33
21	Glomus mosseae	5.72	50
22	Glomus multicaule	0.16	8.33
23	Glomus pustulatum	0.81	8.33
24	Scutellospora reticulata	0.16	8.33
25	Sclerocystis rubiformis	0.16	8.33

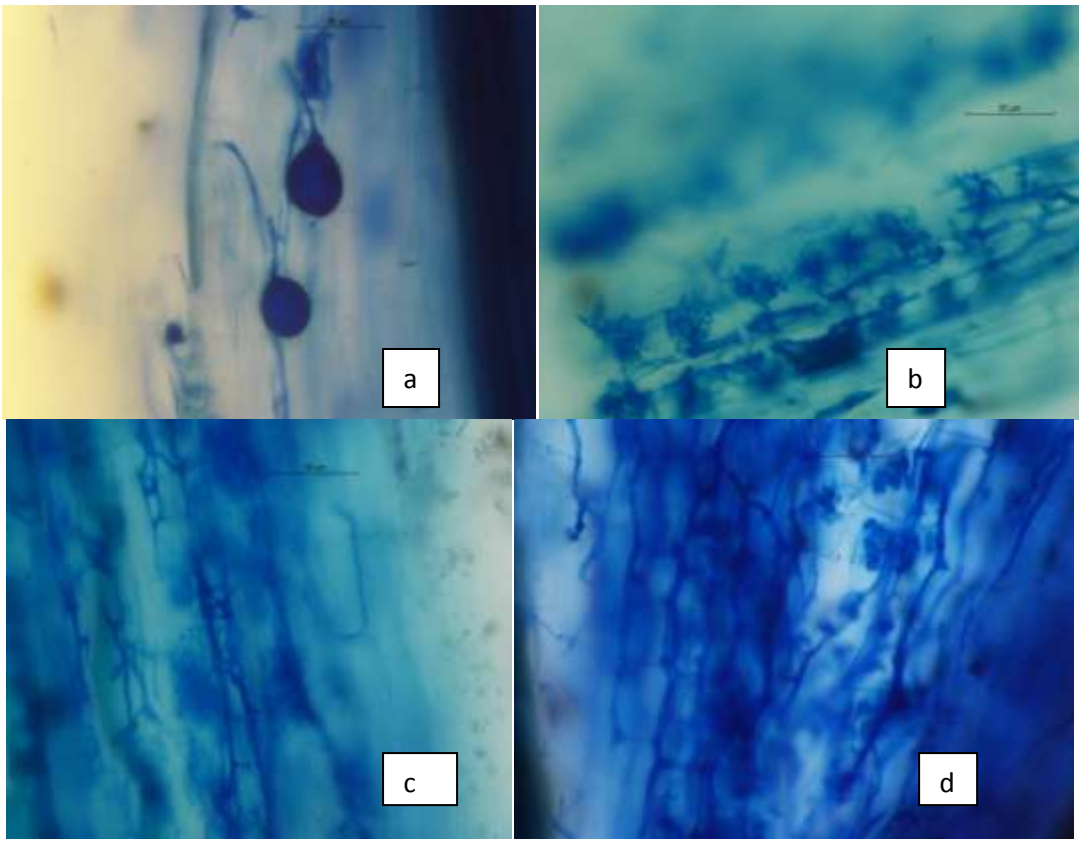

Fig-a= Vesicles of Parthenium hysterophorus L., b= Arbuscules of Parthenium hysterophorus L. c = Arbuscules of Clerodendrum infortunatum Linn., d= Arbuscules of Citrus lemon (L)

Fig – A = Acaulospora spinosa, B = Glomus fasiculatum ,C = Glomus intraradices, D=Spore in spore syndrome

V. Discussion

Glomus species was the most commonly found species in all the plants. Similar results were reported by (Beena et al. 2000 ; Bhuvaneswari 2010) . Acaulospora sp. were also frequently observed. The hyphae of AM fungi play an important role in the formation and stability of soil aggregates and contribute to the composition of plant community structures (Smith and Read,1997). Arbuscular mycorrhizal fungi have been described as'keystone mutualists' in ecosystems due to their unique position at the root-soil interface (Kumar et al. 2010). It is well established that most of the naturally growing plants are mycorrhizal and they are completely reliant on these symbionts for their nutritional and physiological needs. In this context the present research effort was made to understand the distribution, diversity, colonization rates, abundance and isolation frequency of AM Fungi among the commonly available herbaceous plants from J. P. University Campus, Chapra. A total of 25 AMF morphotypes were recovered from 12 herbaceous plants which is considered to be a significant observation when compared to other previous observations of AMF association with plants (Rajkumar and Sunilkumar 2011; Muthukumar et al. 2001). It is important to note that all the plants screened were mycorrhizal. Colonization rates and spore densities also differed among different plant families. Increased spore density did not show increased colonization rates in many herbaceous plants and vice versa. Therefore, there is no correlation between spore density and per cent colonization in the present study. Arbuscular mycorrhizal fungal spores associated with rhizosphere soil but no definite correlation could be established between per cent colonization and spore density, which is in agreement with the findings of Kalita et al. (2002). This could be due to the fact that arbuscular mycorrhizal fungal sporulation is dependent on a wide range of host fungal and environmental factors, and their germination potential varies at different times of the year (Tommerup 1983; Koske and Gemma 1988). In the present study the genus Glomus and Acaulospora were the dominant mycospecies in the roots of herbaceous plants, the possible reasons for the predominance of Glomus species were that spores of Glomus species have different temperature and pH preferences for germination (Wang et al.1997) and Acaulospora species are often associated with acidic soils (Morton 1986; Abbott and Robson 1991). The AM structures , i.e, arbuscules, hyphal coils and vesicles are the sites of storage and exchanging nutrients between fungi and hosts (Bentivenga et al. 1992 and Smith et al. 1989), therefore , there were high AM colonization rates in these growth seasons. AM fungal

colonization is known to depend on soil moisture and P availability (Ruotsalainen et al. 2002; Wang et al. 2010), and physiology, growth rate and turnover of plant roots (Lugo et al. 2003). Based on RA and IF, Glomus and Acaulospora were the dominant genera and Glomus fasiculatum , Glomus intraradices and Glomus claroideum were the dominant species. Bever et al. (1996) reported that Glomus and Acaulospora species usually produce more spores than Gigaspora and Scutellospora species with in the same environment. Because of their smaller spore size, Glomus and Acaulospora species require less time to sporulate (Hepper 1984) and are therefore more adaptive in adjustment of sporulation pattern in varied environmental conditions (Stutz and Morton 1996). Stutz et al. (2000) reported that Glomus species are known to be widely distributed and commonly found in different ecosystems and geographical regions.

VI. Conclusion

The present study confirms the occurrence of AM Fungal in the herbaceous plants of J. P. University Campus, Chapra Bihar. The mycorrhizal fungal are major impact on host plants even under any environmental condition and most important in the biodiversity. All the plant species studied were mycorrhizal ; and the dominant genera were Glomus and Acaulospora. The most relatively abundance species was Glomus intraradices and the maximum isolation ferquency was recorded for Glomus fasiculatum .

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